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Research Article

A case of leucosis in *Heptapterus mustelinus* (Siluriformes, Heptapteridae) among populations of streams in southern Brazil. Has leucosis in *Heptapterus mustelinus* an adaptive value in shaded streams?

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Abstract

Fish populations in environments with a high degree of geographic isolation may be prone to mutations expressed in the phenotypes. These mutations may be related to color pattern, forming leucistic individuals. This work aims to register and to describe possible mechanisms that influence this mutation. Additionally, the study compares other morphometric variations among different populations and leucistic individuals of *Heptapterus mustelinus*. A total of four leucistic individuals were collected in a small shaded stream, highly segmented by rapids and waterfalls. The biometric analyses showed no significant morphological differences when compared to other populations of the same ecoregion. The selection of leucism may be directly related to the sampled environment, since the leucistic specimens occurred in a shaded stream with dense vegetation cover. Low occurrence of predatory species of fish can be an important point to maintain the characteristic. Consequently, predation may not exert a negative selective pressure on leucistic individuals.

Key words: Catfish, color patterns, costal basin, Neotropical

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Introduction

The color of an individual plays a critical role in its survival and reproduction. Pigmentation patterns are essential for intraspecific communication, species recognition, partner choice, and interspecific communication with predators or prey, including warning color, mimicry, and camouflage (Protas and Patel 2008). Color-altering categories may be a congenital or hereditary disorder that reduces or increases pigment production in skin cells, hair, and eyes (Westerman and Birge 1978; Dorp 1987; van Grouw 2012)

Changes in skin color of individuals are defined according to the pattern that is expressed by the phenotype. Albinism is a mutation that causes the absence of color in epithelial tissue and eyes (Reum et al. 2008). Xanthochromism or xanthism is characterized by yellow-orange-red pigmentation, due to unusual

and relatively high levels of xanthophores in the individual's skin (Lewand et al. 2013; Muto et al. 2016). Melanism that is characterized by excess dark pigmentation (Corbalán et al. 2018), and leucism is a phenotype that does not express pigmentation in the epithelial tissue, but the eyes are of normal color (Bechtel 1995).

Specimens with altered color patterns are recorded in all vertebrate groups: mammals (Smírnov 2014; Toledo et al. 2014; Utzeri et al. 2016), birds (van Grouw 2012; Moller et al. 2013), reptiles (de Noronha et al. 2013), amphibians (López and Ghirardi 2011; Toledo et al. 2011) and fish (Lara-Mendoza and Guerra-Jiménez 2018).

For the fish group, mutations of loss of phenotypic characteristics such as color and eyes were studied in *Astyanax mexicanus* (Blind Cavefish), which has colonized underground environments several times throughout evolutionary history. They colonized dark and cavern-like environments and eventually suffered mutations of loss of color and eyes (Coghill et al. 2014). However, albinism or leucism is recorded in surface fish such as *Hyporthodus drummondhayi* (Schwartz 1978), *Scoliodon laticaudus* (Veena et al. 2011), *Lebiasina bimaculata* (Nugra et al. 2018). Additionally, Lara-Mendoza and Guerra-Jiménez (2018) reported a list of Siluriformes species that exhibit pigmentation mutations, albinism and leucism, indicating that this mutation is more frequent in individuals of this order.

The ichthyofauna of the Neotropical region is composed of more than 6,000 fish species (Reis et al. 2003). The authors of this study are aware of only 15 cases of albinism, hypomelanism and leucism. Siluriforms lead this list with 13 records (Nobile et al. 2016; Manoel et al. 2017). However, studies that discuss the behavioral and ecological influences of these pigmentation mutations are still scarce (Slavík et al. 2016) , only ecological notes (Sazima and Pombal 1986) and observations on sexual selection (Karadal and Güroy 2015) are published.

The genus *Heptapterus* is widely distributed in rivers and streams in South America (Aguilera et al. 2011) and consists of ten species (Deprá et al. 2022), *H. bleekeri* Boeseman, 1953, *H. tapanahoniensi* Mees, 1967 and *H. tenuis* Mees, 1986 in northern South America; *H. fissipinnis* Miranda Ribeiro, 1911, *H. multiradiatus* Ihering, 1907 and *H. stewarti* Haseman, 1911, *H. carmelitanorum* Depra et al. 2022 in southeastern Brazil, *H. sympterygium* Buckup, 1988, *H. mbya* Azpelicueta et al., 2011 and *H. mustelinus* (Valenciennes, 1835) in southern South America.

The species *H. mustelinus* (Valenciennes, 1835) can be generally determined by the following characteristics (Sensu Bockmann, 1998): medium-sized catfish, up to 262.9 mm in maximum standard length, elongated body, depressed head, 18–23 rays in the anal fin, more than 26 rays in the lower lobe of the caudal fin, latero-posterior portion of the trunk with pigments aligned along the myosepts, marking the border between the myomeres. Bockmann (1998) describes their colour as earth brown.

The general objective of this study is the morphometric comparison of the leucistic individuals with normal colored individuals of the same stream and of other river basins and to discuss possible adaptive values of leucistic forms in relation to their specific environment.

Material and methods

Study areas and sampling sites

The work was carried out in the Serra Geral (29°08'02"S, 49°59'40"W) (PNSG) and Aparados da Serra National Parks (29°11'30"S, 50°05'51"W) (PNAS) and their buffer zones located northeast of the states of Rio Grande do Sul and south of Santa Catarina, Brazil. The parks are located southwest of the geological formation of the Paraná basin, with a geological age range between 120–135 Ma. Fish collections were performed on August 14 and 16, 2015 and April 30, 2016. The collection was part of a larger study to establish the species lists of the Conservation Units and the adjacent areas. *Heptapterus mustelinus* was captured at the site named Vista Alegre Waterfall (29°07'46.8"S, 49°56'20.1"W), which belongs to the Mampituba River basin that divides the states of Rio Grande do Sul and Santa Catarina, in the south of Brazil (Fig. 1). All fishes were sampled by electric fishing (750 V unpulsed DC; EFKO, Germany). The captured specimens were euthanized with Eugenol solution (Lucena et al. 2013) and fixed in 10% formaldehyde. All individuals were identified, fixed and deposited in the fish collection at the Laboratory of Ichthyology da Universidade do Vale do Rio dos Sinos (UNICTIO). Fishes sampled in the Mampituba basin were compared to population of six additional river basins: Araranguá River (coastal drainage), and dos Sinos, Caí, Gravataí, Jacuí, and Piratini Rivers (Laguna dos Patos system). All groups are distributed by regions 334 (Patos system) and 335 (coastal drainage) according to Abell et al. (2008). Both drainages show a high percentage of endemism between 21% and 39%

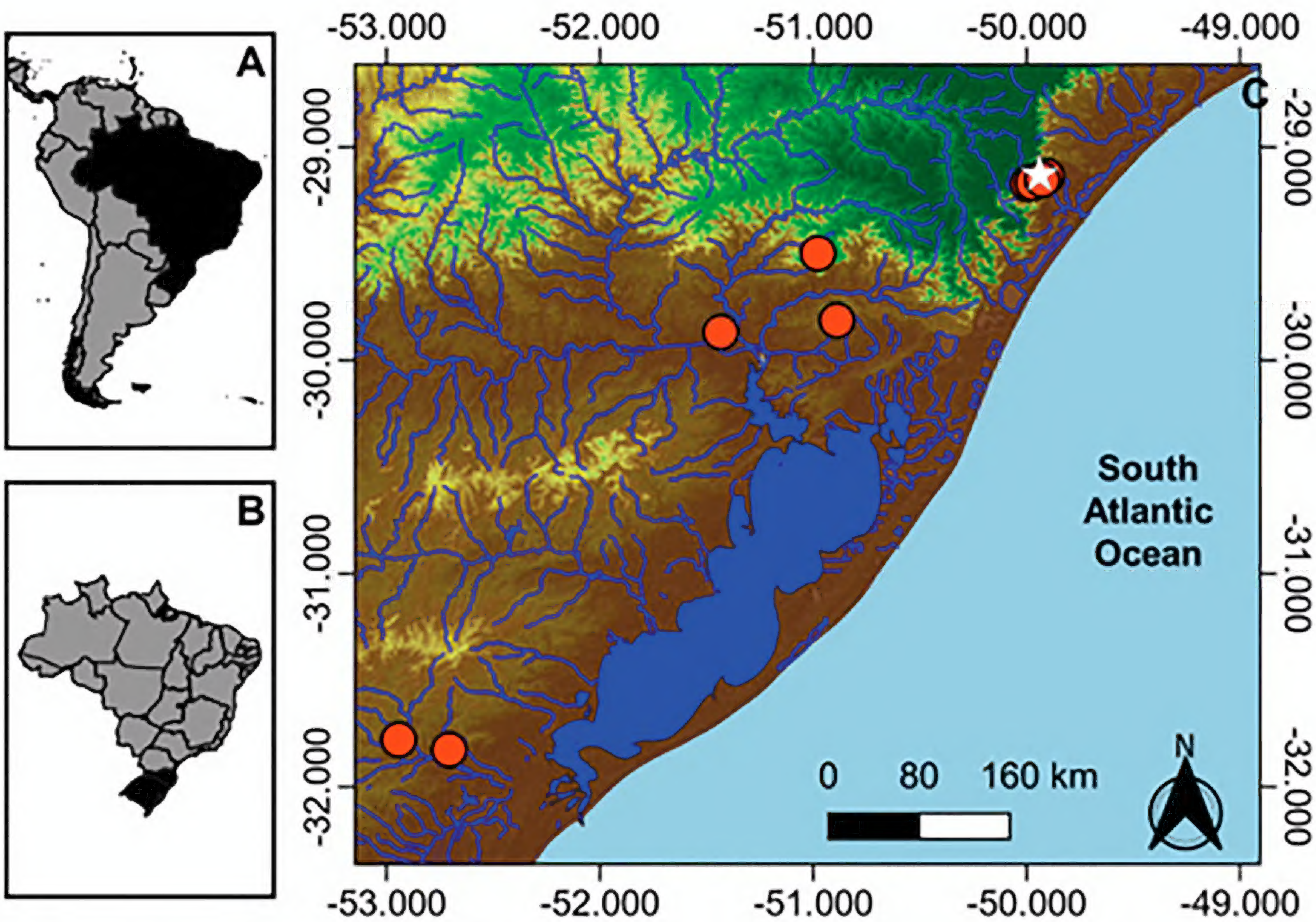

Figure 1. **A** South America with Brazil **B** Brazil with the Rio Grande do Sul State **C** study area: blue lines: stream network; white star: sample site of leucistic individuals; red circles: *H. mustelinus* populations, which were compared morphologically to the leucistic individuals.

Morphometry procedure

All *Heptapterus mustelinus* were measured as proposed by Bockmann (1998). Individuals of the other basins were also measured using lots deposited in the fish collection of Universidade do Vale do Rio dos Sinos. The morphometric characters were analyzed including two conventional measurements, standard length (SL) and head length (HL), 25 measures related to SL and seven to HL.

Statistical analysis

Data were log transformed and subjected to principal component analysis (PCA) to assess phenotypic differences among to assess phenotypic differences among four leucistic individuals, normally coloured fishes of the same River basin (Mampituba River, n= 16) and populations of six other basins (Araranguá River, n= 3; Sinos River, n= 4; Caí River, n= 2; Gravataí River, n= 2;Jacuí River, n= 2; Piratini River, n= 5). Individual scores from PCA were used to construct a scatterplot to reveal the specimen groupings. The analysis evaluates the relationships among populations according to their proximity in the space defined by the components. The statistics were performed in Past 4.1 software.

Results

At Vista Alegre in the Mampituba basin a total of 39 *H. mustelinus* were captured. Of these, 35 were normally colored (Appendix 1: table 1) and four individuals displayed leucism (Fig. 2) (Appendix 1: table 2). At the same sampling site five additional fish species occurred in sympatry with *H. mustelinus*. A total of 32 measurements were performed on each individual (Appendix 1: table 1). The comparison of normal and leucistic individuals from Mampituba River by PCA showed that the morphometric measurements of the normal colour individuals overlap the leucistic individuals (Fig. 3). No significant differences were detected, but the measurements that showed the greatest variation were interdorsal length to PC2, with greater contribution (Eigenvalue = 25.75 and 0.52% of variation) and the interdorsal-dorsal length, preadipose distance to PC3 with contribution lower contribution (Eigenvalue = 17.00 and 0.24% of variation).

The comparison of leucistic individuals from the Mampituba River and the other individuals of the species, did not show differences in morphometry. Leucistic individuals maintained the morphometric pattern of the normal coloured *H. mustelinus* (Fig. 3). The measurements that showed the highest variation were standard length, caudal peduncle length, pectoral fin length and the interdorsal distance for PC2 with the greatest contribution (Eigenvalue = 27.75 and 0.52% of variation) and the pre-adipose distance, length of the base of the adipose fin and the interdorsal distance for p PC3 with the smallest contribution (Eigenvalue= 17.00 and 0.24% of variation).

At the Vista Alegre waterfall, where the leucistic individuals of *Heptapterus mustelinus* were captured, *Characidium pterostictum* (25 individuals), *Pareiorhaphis hypselurus* (69 ind.) *Cambeya aff. cubataonis* (1 ind.) and *Rineloricaria aequalicuspis* (2 ind.) occurred sympatrically.

Figure 2. Lateral view of *Heptapterus mustelinus* from the coastal streams of the Mampituba River **A** leucistic individual in life (54.74 mm SL), UNICTIO 2396 **B** leucistic individual fixed (112.8mm SL), UNICTIO 2035-2 **C** Individual with regular pattern color (87.7 mm SL), UNICTIO 2395-9.

One leucistic individual was an adult with 112.8 mm SL, and three were juveniles with a mean SL of 62.5 mm (SD = 15.20). The results of the PC2 and PC3 axis show that the leucistic individuals fit into the general *H. mustelinus* measurement pattern (Fig. 3). PCA showed additionally that the fish were grouped by species pattern measurement, and that the populations of the Mampituba River encompass all groups.

Material examined

Brazil: UNICTIO 051, 4 ind., 50.44–76.95 mm SL, rio dos Sinos, Schulz U. H.; UNICTIO 796, 4 ind., 63.82–71.78 mm SL, stream within the Fazenda São José, Pedro Osório, RS, 31°46'47.7"S, 52°56'34.7"W, 7 Sep 2012, Col. Pereira, B. e Bartzen, C.; UNICTIO 874, 1 ind., 60.53 mm SL, Ponte das Carretas, Capão do Leão, RS, 31°49'38.4"S, 52°42'21.7"W, 20 Oct 2012, Col. Pereira, B., Cavalet, E.; UNICTIO 1245, 2 ind., 61.92–67.7 mm SL, arroio Demétrio, Morungava, Gravataí, RS, 29°48'47.4"S, 50°53'21.2"W, 11 Apr 13, Col. Lehmann, P., Arim, A., Diene, L., Bono, A., Borsoi, J.; UNICTIO 1375, 2 ind., 46.49–76.87 mm SL, Sítio do Alcione Demétrio, bacia do rio Jacuí, Triunfo, RS, 29°52'05.7"S, 51°26'02.8"W, 20 Sep 2013, Col.

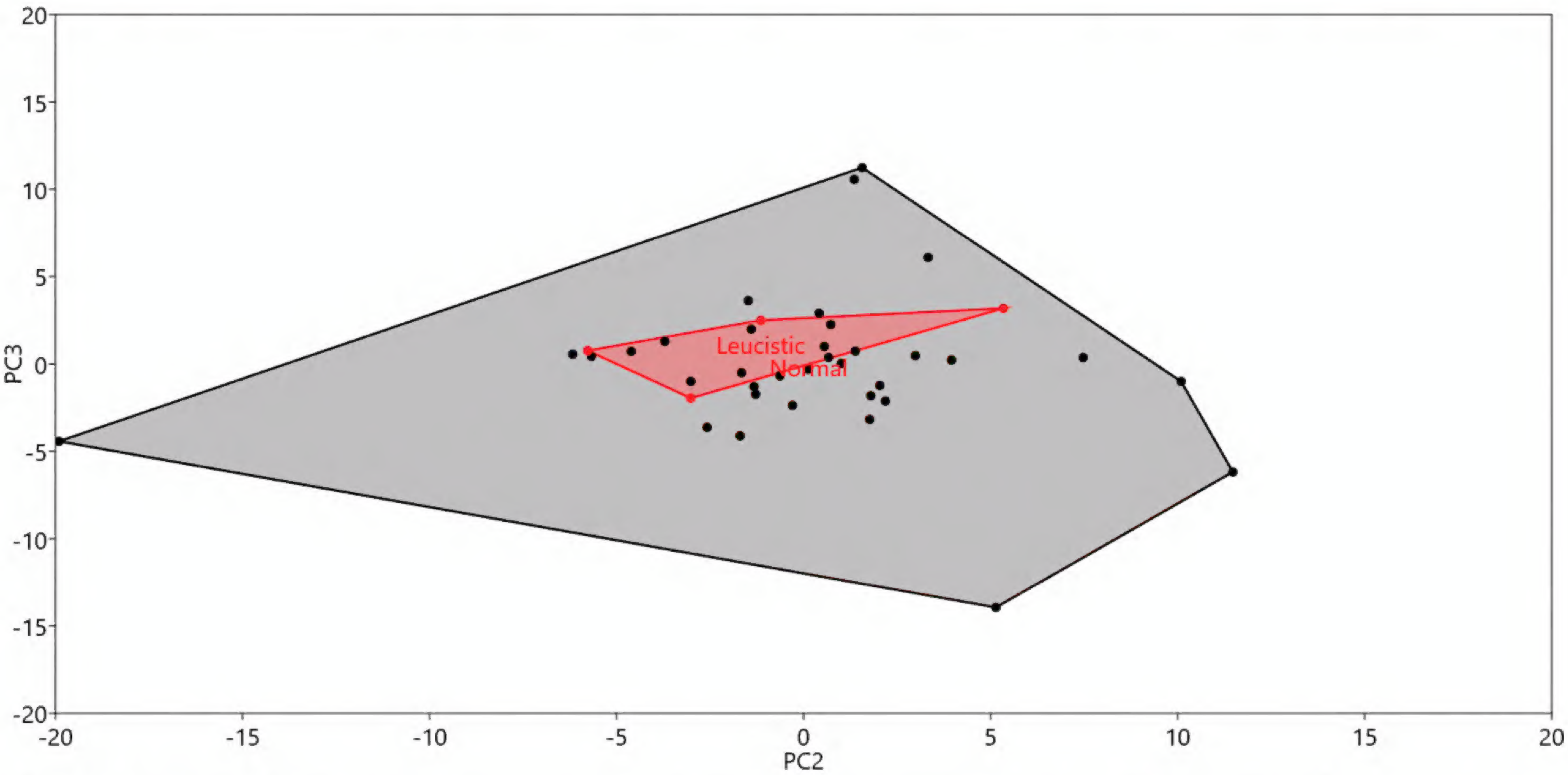

Figure 3. PCA axis with the measurements taken on *Heptapterus mustelinus*, leucistic individuals (red polygon) in comparison with normal colored individuals (black polygon).

Brites, P., Ramalho, G.; UNICTIO 1834, 2 ind., 110.59–127.18 mm SL, tributary of rio Cadeia, 29°29'56.82"S, 50°58'40.68"W, Col. Bartzen, C.; UNICTIO 2074, 3 ind., 95.9–115.44 mm SL, arroio Molha Coco (bacia Mampituba), Praia Grande, SC, 29°10'17.6"S, 49°59'37.5"W, 3 Ago 2014, Col. Lehmann, P.; Schulz, U.; Silveira, L.; Bono, A.; Ferraz, M. & Santos, L.; UNICTIO 2109, 2 ind., 91.21–95.41mm SL, arroio Malacara, above the bridge (Bacia Mampituba) - Praia Grande/SC, 29°10'08.8"S, 49°58'17.2"W, 16Ago2015, Col. Lehmann, P.; Schulz, U.; Silveira, L.; Bono, A.; Ferraz, M. & Santos, L; UNICTIO 2125, 1 ind., 71.5mm SL, arroio Cachoeira (Bacia Mampituba) - Praia Grande/SC, 29°08'11.6"S, 49°54'21.1"W, 16 Ago 2015, Col. Lehmann, P.; Schulz, U.; Silveira, L.; Bono, A.; Ferraz, M. & Santos, L.; UNICTIO 2151, 1 ind., 56.60 mm SL, arroio Cachoeira (Bacia Mampituba) - Praia Grande/ SC, 29°08'11.6"S, 49°54'21.1"W, 16 Ago 2015, Col. Lehmann, P.; Schulz, U.; Silveira, L.; Bono, A.; Ferraz, M. & Santos, L.; UNICTIO 2395, 4 ind., 87.0–202.01mm SL, Vista Alegre Waterfall, rio Mampituba Basin - Praia Grande/ SC, 29°07'46.8'S, 49°56'20.1"W, 30Apr2016, Col. Lehmann, P.; Bono, A.; Santos, L.; Silveira, L. & Haas, M.; UNICTIO 2457, 3 ind., 102.76–147.13 mm SL, Tres Rios (Bacia Ararangua) - Jacinto Machado/RS, 29°3'27.29"S, 49°57'45.97"W, 06Jun2016, Col. Ferraz, M. & Santos, L.; UNICTIO 2483, 1 ind., 107.62 mm SL, Arroio Molha Coco, bridge SC 290, Mampituba Basin - Praia Grande/SC, 29°11'42.7"S, 49°57'54.2"W, 26Ago16, Col. Schulz, U.; Santos, L.; Ferraz, M; Neumann, M. & Haas, M.; UNICTIO 2506, 4 ind., 51.51–69.07 mm SL, arroio Três Irmãos - rio Mampituba Basin- Praia Grande/SC, 29°9'11.9"S, 49°55'34.6"W, 26Ago2016, Col. Schulz, U.; Ferraz, M; Neumann, M. & Haas, M.; UNICTIO 2542, 1 ind., 86.95mm SL, Passagem molhada Malacara, Araranguá basin - Praia Grande/SC, 29°10'09.5"S, 49°58'16.9"W, 24Ago2016, Col. Ferraz, M; Neumann, M. & Haas, M.; UNICTIO 2035, 2 ind., 80.09–112.85mm SL, Vista Alegre Waterfall (Mampituba basin) - Praia Grande/SC, 29°07'46.8"S, 49°56'20.1"W, 14Ago2015, Col., Lehmann, P.; Silveira, L.; Bono, A.; Ferraz, M. & Santos, L.; UNICTIO 2134, 1 ind., 52.86mm SL, Vista Alegre Waterfall, downstream (Mampituba basin) - Praia Grande/SC,

29°07'46.8"S, 49°56'20.1"W, 16Ago2015, Col., Lehmann, P.; Schulz, U.; Silveira, L.; Bono, A.; Ferraz, M. & Santos, L.; UNICTIO 2396, 1 ind., 54.74mm SL, Vista Alegre Waterfall, Rio Mampituba basin - Praia Grande/ SC, 29°07'46.8'S, 49°56'20.1"W, 30Apr2016, Col. Lehmann,P.; Bono, A.; Santos, L.; Silveira, L. & Haas, M.

Discussion

The Heptapteridae family is widely distributed in South American streams. Its habits are nocturnal (Bockmann and Guazelli 2003). Their brown earthy coloration is a characteristic to avoid predation in the presence of fish or piscivorous birds (Uieda and Motta 2007). Albinism or leucism can be caused by factors such as exposure of eggs or adults to heavy metals, which may affect offspring (Oliveira and Foresti 1996) or mutations that reduce metabolism and melanin production (Leal et al. 2013).

In this study, heavy metal contamination is not a plausible hypothesis, since the sampling site is downstream of an environmentally preserved area near to the National Park Aparados da Serra, Rio Grande do Sul state. The stretch where the leucistic individuals were sampled is characterized by rapids and waterfalls that are geographical barriers for genetic flow among sub-populations (Buckup 1999). It is possible that the mutation that caused leucism is favored by the structure of the environment.

Leucism and albinism are commonly reported in catfish (Lara-Mendoza and Guerra-Jiménez 2018), but they occur as well in other orders. Karadal and Güroy (2015) tested sexual selection between normal and albino individuals of *Chindongo socolofi* (Cichliformes) and found that albino individuals were less selected. However, Hattori et al. (2010) found a leucistic individual of *Tachysurus ichikawai* in the Toyo River, Japan. This leucistic individual was crossed with a normal specimen and the author observed that the lack of skin color remained in 25% of the offspring, maintaining the Mendelian genetic pattern.

The shaded environment where the leucistic individuals occur in the Mampituba River may further reduce the effect of predation. However, during reproduction, conspicuous leucistic individuals may be positively selected and increase their abundance in the population.

The coloration of leucistic or albino individuals is easily perceived by predators, thus making them easy prey which can reduce the abundance of leucistic individuals in the population (Sazima and Di Bernardo 1991; Slavík et al. 2016). However, in our samples the leucistic individuals co-occurred with small, insectivorous species, or with those that feed preferentially on invertebrates and periphyton on the available substrate. No predator fish were present. Other vertebrate predators like the South American racoon (*Procyon cancrivorus*) (Gatti et al. 2006; Quintela et al. 2014) or birds of the kingfisher family (Alcedinidae) are visually orientated. Their predation success on *H. mustelinus* most probably is low due to reduced optical conditions in the shaded areas caused by the dense vegetation.

The lack of pigmentation may not only be related to hereditary diseases, but it could also be due to the lack of tyrosine in the diet which causes a deficiency in the production of melanin (van Grouw 2012). However, the degree of geographic isolation may help abnormal features to prevail in a population, since the isolated population has less or no genetic flow with other populations (Sazima and Pombal 1986). The individuals which were analyzed inhabited a stream running

in a gallery forest with small caves between the rocks. The environment favors individuals that can shelter themselves during the illuminated period of the day.

According to the results of the PCA, it can be observed that leucistic individuals have a more elongated anterior region of the body compared to normal individuals. However, for all populations it can be observed that the leucistics maintain the species pattern. Only *H. mustelinus* from the Araranguá River showed differences in relation to the other populations. However, it did not affect the results of the morphometry of leucistic individuals. The variations presented here may be related to affinities toward environments between or under rocks, thus facilitating their access to cavernlike environments.

The fact that individuals with leucism of two size classes were recorded reinforces the possibility that they are being selected more easily. Another fact is that another individual was collected with this characteristic during the second year of collection, but of smaller size. This demonstrates that the mutation may be a genetic factor, since part of the individuals were previously removed from the population, including the leucistic ones, and even so the pattern kept appearing.

The arguments presented here support the hypothesis that mutations such as leucosis cannot always be considered as deleterious or unfavorable to populations. Fish exhibiting leucism, with populations restricted to streams with a certain degree of geographic isolation and with few sympatric species, may be favored and maintained as in the case of *H. mustelinus*.

Future studies to understand how this characteristic is maintained in fish should be performed, because these reports are being registered frequently in the Neotropical region. That may possibly have environmental influences, for example, on stream restricted environments.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: PLA, UHS. Data curation: UHS, PLA, MF. Formal analysis: PLA, UHS, MF. Investigation: MF, PLA, UHS. Methodology: PLA, MF. Project administration: PLA. Software: MF, UHS, PLA. Supervision: CASL, PLA. Validation: CASL, MF, PLA. Visualization: MF, PLA. Writing - original draft: MF, PLA. Writing - review and editing: UHS, PLA, CASL.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Appendix 1

Table A1. Morphometric data of *Heptapterus mustelinus*, n = 35 from the Mampituba, Piratini, and Araranguá Rivers, Brazil. SD = Standard Deviation.

	N	Min	Max	Mean	SD
Standard length (mm)	35	46.49	202.01	–	–
Percent of Standard Length					
Preadipose distance		46.9	61.6	55.4	2.90
Prepectoral distance		14.5	22.8	18.0	1.86
Prepelvic distance		32.4	40.1	36.4	1.81
Preanal distance		55.0	64.7	60.1	2.59
Body depth		7.7	13.6	10.8	1.54
Peduncle depth		4.6	6.9	6.0	0.54
Peduncle length		13.6	28.4	18.5	2.65
Body width		9.9	14.4	12.1	1.36
Dorsal-fin base		7.8	11.4	9.3	0.81
Anal-fin base length		18.7	27.5	22.1	1.80
Pectoral-fin length		2.5	13.9	10.5	2.13
Pelvic-fin length		6.9	13.0	11.0	1.40
Unbranched dorsal-fin ray length		5.6	16.7	8.5	1.79
Dorsal-fin length		9.9	19.8	15.4	1.78
Unbranched pectoral-fin ray length		4.6	8.6	7.1	1.17
Pectoral-pelvic distance		15.0	20.2	17.1	1.12
Pelvic-anal origin distance		19.2	28.3	22.5	1.89
Adipose-fin depth		1.5	5.2	3.4	0.86
Adipose-fin base length		39.0	49.8	44.1	2.56
Interdorsal length		4.0	19.8	11.7	3.44
Head lentgth		15.1	20.8	18.3	1.38
Dorsal-fin origin-hypural plate		60.7	80.5	65.8	3.35
Anal-fin origin-hypural plate		36.5	50.8	40.4	3.03
Percent of Head Length					
Snout length		27.0	44.3	34.7	3.31
Orbital diameter		10.5	22.5	16.5	2.75
Head width		68.2	87.5	76.1	5.73
Mouth width		42.1	72.4	52.1	5.72
Upper prognathism		0.8	14.1	3.9	2.41
Postorbital distance		43.1	60.7	54.1	4.00
Interorbital width		15.7	30.2	22.0	2.90

Table A2. Morphometric data of *Heptapterus mustelinus*, n = 4 leucistic from the Mampituba, Brazil. SD = Standard Deviation.

	N	Min	Max	Mean	SD
Standard length (mm)	4	52.86	112.85	75.14	–
Percent of Standard Length					
Preadipose distance		53.8	57.7	56.2	1.63
Prepectoral distance		15.3	19.1	17.7	1.64
Prepelvic distance		34.6	37.8	36.5	1.39
Preanal distance		57.2	61.6	59.5	2.17
Body depth		8.7	10.0	9.5	0.58
Peduncle depth		4.6	6.3	5.6	0.76
Peduncle length		16.1	20.9	18.7	2.47
Body width		9.9	12.7	11.4	1.37
Dorsal-fin base		8.4	10.7	9.6	0.96
Anal-fin base length		18.7	22.1	20.9	1.48
Pectoral-fin length		10.2	13.9	11.5	1.73
Pelvic-fin length		10.1	13.0	11.6	1.46
Unbranched dorsal-fin ray length		8.2	9.7	9.1	0.64
Dorsal-fin length		15.7	19.8	17.2	1.86
Unbranched pectoral-fin ray length		7.4	8.6	8.2	0.58
Pectoral-pelvic distance		16.3	20.2	17.6	1.80
Pelvic-anal origin distance		21.8	24.4	22.8	1.14
Adipose-fin depth		1.5	3.2	2.6	0.78
Adipose-fin base length		41.0	47.7	44.1	2.95
Interdorsal length		9.4	14.2	12.8	2.27
Head length		17.4	20.8	18.8	1.48
Dorsal-fin origin-hypural plate		64.1	66.2	65.3	1.04
Anal-fin origin-hypural plate		37.7	40.7	39.3	1.23
%Head length		12.5	24.9	18.2	5.10
Percent of Head Length					
Snout length		33.2	38.1	36.0	2.23
Orbital diameter		10.5	17.0	13.9	2.65
Head width		68.5	70.8	69.7	1.04
Mouth width		48.4	54.8	51.5	2.61
Upper prognathism		0.8	1.8	1.3	0.52
Postorbital distance		43.1	52.4	48.7	4.15
Interorbital width		18.5	20.8	19.2	1.12
Snout-anterior nostril distance		7.1	12.4	9.1	2.34
Internarial distance		17.1	23.5	20.7	2.67
Head depth		43.1	49.7	45.8	2.83